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Human bancroftian filariasis – a role for antibodies to parasite carbohydrates

M. C. MOHANTY, A. K. SATAPATHY, P. K. SAHOO & B. RAVINDRAN* *Division of Immunology, Regional Medical Research Centre, ICMR, Bhubaneswar, India*

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SUMMARY

Studies on immune responses to parasites have been undertaken in filariasis with a view to understand protective immunity, pathogenesis of the disease process and mechanisms of immune deviation. However none of the investigations conducted so far on antibody responses have addressed the issue of immunogenicity of filarial carbohydrate antigens in human lymphatic filariasis. In this communication we report details on relative protein and carbohydrate contents of various developmental stages of filarial parasites and antibody responses to filarial proteins (Fil.Pro) and carbohydrates (Fil.Cho) in different clinical spectrum of human bancroftian filariasis. As expected, antibodies of IgM and IgG2 subclass recognized primarily Fil.Cho while IgG4 filarial antibodies recognized exclusively Fil.Pro. Reactivity of IgG3 to Fil.Cho was similar to that of IgG2 while IgG1 more readily recognized Fil.Pro than Fil.Cho. The IgG2 and IgG3 antibodies to Fil.Cho were found to be significantly more in patients with chronic filarial disease and in endemic normals when compared with microfilariae (mf) carriers while IgG4 antibodies to Fil.Pro were significantly more in mf carriers. The dichotomy in reactivity of filarial IgG2, IgG3 and IgG4 was dependent on active filarial infection as indicated by presence of circulating filarial antigen (CFA). Individuals with CFA were found to possess significantly more IgG4 to Fil.Pro than those without CFA while IgG2 and IgG3 levels to Fil.Cho was significantly more in CFA negative subjects when compared to those with CFA. Although IgG1 reacted more readily with Fil.Pro, unlike IgG4, their levels were significantly more in CFA negative subjects when compared to those with active filarial infection. Absorption of sera with phosphorylcholine (PC) resulted in no significant loss of reactivity to Fil.Cho indicating that most of the anticarbohydrate antibodies were recognizing non-PC determinants in human filariasis. Elevated levels of IgG2 and IgG3 antibodies to Fil.Cho in individuals free of filarial infection indicate a possible role for carbohydrate antigens in induction of protective immunity in human filariasis.

Keywords human filariasis *Wuchereria bancrofti* antibodies to carbohydrates IgG subclass circulating filarial antigen.

INTRODUCTION

Lymphatic filariasis, a debilitating helminthic disease affects about 120 million people worldwide. The parasites survive for several years in mammalian hosts, often without causing overt clinical manifestations and the factors that contribute to parasite survival are largely unknown. Antibody as well as T-cell responses in human and experimental filariasis have been undertaken with a view to understand the nature of acquired immunity, pathogenesis and mechanisms of immune evasion/deviation that could assist in survival of these extracellular parasites for several years in the lymphatics [1–4]. In human filariasis, microfilariae(mf) carriers characteristically produce higher antifilarial IgG4 than other subclasses of filarial specific IgG. T-cell response in mf carriers is characterized by very low production of IFN-γ by filarial specific T-cells. Patients with chronic filariasis (elephantiasis and hydrocele) and subjects without demonstrable filarial infection (as shown by absence of circulating filarial antigen, CFA, a product of adult worms) on the other hand are found to possess lower levels of IgG4 filarial antibodies and filarial specific T-cells from such individuals release higher levels of IFN-γ in response to filarial antigens *in vitro* [3,4]. For all the investigations on antibody responses in human filariasis only crude extracts of infective larvae, adult stage parasites and/or excretory-secretory antigens have been used although in recent years there have been attempts to use recombinant protein antigens [5,6]. Curiously however, no attempt has been made so far to study the antibody responses to filarial carbohydrates in experimental or human filariasis. Carbohydrate antigens have been proposed to act as decoy

Correspondence: Dr B. Ravindran, Deputy Director, Division of Immunology, Regional Medical Research Centre, Indian Council of Medical Research, Chandrasekharpur, Bhubaneswar-751 023, India.
E-mail: immunol@dte.vsnl.net.in

antigens for diverting the immune response away from protective protein epitopes in helminthic infections and antibodies to carbohydrates have been suspected to block the effector immune function in Schistosomiasis [7,8]. In view of these and other reports on the study of immunogenicity of parasite carbohydrates in other helminthic diseases such as Trichinellosis and Hydatidosis [9,10] we have attempted to address the issue of immunogenicity of filarial carbohydrates in human Bancroftian filariasis.

MATERIALS AND METHODS

Collection of human blood samples for sera

Clinical examination and nocturnal blood survey was conducted in four areas of Puri and Nayagarh district of Orissa State, India, which are highly endemic for Bancroftian filariasis [11]. Parasitological examination of individuals was done by microscopic examination of Giemsa stained finger prick blood smear (20 μl) obtained by night blood survey. The individuals were examined clinically for presentation of acute or/and chronic disease manifestations of lymphatic filariasis. The following inclusion/exclusion criteria were followed for classification:

1 Chronic filariasis. Patients presenting with persistent ($>$ 5 years) Grade III nonpitting oedema, nonreversible on elevation with thickened skin [12]; the prevalence of filarial antigenemia (CFA) was about 17% in this group [11].

2 Microfilariae carriers. Asymptomatic individuals with circulating microfilariae.

3 Endemic normals. Asymptomatic, amicrofilaraemic individuals without Cirulating filarial antigen.

About 5 ml blood was collected from volunteers who consented to give blood and sera were separated and kept frozen at – 20°C.

Collection of Setaria digitata

Peritoneal dwelling bovine filarial parasites were collected in PBS-glucose at a local abatoir. Freshly collected adult male and female parasites were transported to the laboratory for preparation of native antigen from which protein and carbohydrate fractions were purified as described below.

Preparation of antigens

Native S. digitata *antigen (Fil-Nat).* A PBS extract of adult female worms of *S. digitata* was prepared and used for the study as native antigen (Fil.Nat). The parasites were washed extensively in PBS and ground in a glass homogenizer and ultrasonicated (Artek Sonic Dismembrator, Model-150, Artek Systems Corp., USA) for 1 minute. The soluble antigen was harvested by microfuging at 2500 $\times$ g for 10 min and kept frozen at $-$ 20°C.

Deglycosylated protein antigen (Fil.Pro). For preparation of protein antigens, Fil-Nat was dialysed overnight in 50 mM Acetate buffer (pH 4·5) and treated with 25 mM sodium meta-periodate (Sigma) for 1 h. The reaction was stopped by treating with 50 mM sodium borohydride for 30 min at RT and the sodium meta-periodate oxidized antigen was dialysed extensively against PBS and kept frozen at – 20°C until further use.

Carbohydrate antigen (Fil.Cho). Carbohydrate fraction devoid of proteins was prepared by addition of 9 volumes of 10% Trichloroacetic acid to 1 volume of Fil.Nat followed by incubation at 4°C for 1 h, microfuged at 2500 $\times$ g for 10 min and the supernatant was dialysed against PBS. Pronase (50 μg/ml final concentration) (Sigma Chemical Co., MI, USA) was added to the carbohydrate rich supernatant, incubated for 1 h at 37°C, boiled for 5 min, microfuged, dialysed against PBS and kept frozen at $-$ 20°C for further use.

Other filarial antigens. Solubulized mf antigens were prepared by ultrasonication of intrauterine mf released in-vitro from adult female worms. Excretory/Secretory (ES) antigens from adult female worms of *S. digitata* were prepared by incubating viable worms in serum free DMEM for 24 h and the spent medium was centrifuged at 1200 $\times$ g for 10 min to pellet mf and the supernatant was concentrated 50 fold using centricon tubes (Amicon, U.K), dialysed against PBS and frozen at $-$20°C.

Preparation of PC-BSA. 2·5 mg of p-aminophenylphosphorylcholine (Sigma) and 1 mg of BSA (Sigma) were taken in 1 ml of PBS and 20 μl of 2·5% glutaraldehyde was added. After 1 h. incubation at room temperature the mixture was dialysed overnight against 150 mM NaCl and the supernatant was collected after microfuging at 2500 $\times$ g and used as PC-BSA. BSA cross-linked similarly with 2·5% glutaraldehyde (BSA-g) was used as control antigen for coating in ELISA as described below. The reactivity and sensitivity of PC-BSA used for detecting PC specific antibodies in immunoassays was established by using a standard MAb to PC kindly provided by Dr T.V.Rajan, University of Connecticut Health Center, Farmington, USA.

Carbohydrate and protein estimation

The protein content in all the parasite antigen preparations were estimated by Lowry's method. The carbohydrate content was quantified using phenol–sulphuric acid assay. Briefly, the samples and standards (different concentrations made upto volume of 100 μl) were mixed with 200 μl of 5% phenol reagent followed by addition of 1 ml of concentrated sulphuric acid. The absorbance was measured at 490 nm after 30 min incubation at RT.

Wuchereria bancrofti *antigen detection assay*

The assay was performed by capture sandwich ELISA according to the manufacturer's recommendations (JCU, Tropical Biotechnology, Townswille, Australia). The sera samples were boiled with EDTA, centrifuged and the supernatants were used for antigen detection. The results were expressed in arbitrary antigen units per milliliter using *O. gibsoni* antigen provided as standard in the kit (cut off = 100 units/ml).

Enzyme linked immunosorbent assay (ELISA)

Fil.Nat, Fil.Pro and PC-BSA. Flat bottomed 96-well polystyrene plates (Corning, NY, USA) were coated with 2 μg/well of the appropriate antigen diluted in PBS, incubated for 5 h at 37°C and then kept overnight at 4°C. For coating Fil.Cho the plates were pretreated with poly l-lysine-hydrochloride (1 μg/well) (Sigma) for 1 h, washed thrice in PBS and incubated with 2 μg/well of antigen at 37°C overnight and at 4°C for 48 h. After blocking with 1% BSA the dilutions of sera in PBS with 0·1% Tween 20 (PBS-T) were incubated for 2 h. Human sera for detection of IgG and IgM were diluted 250 fold and for IgG isotypes the sera were diluted 100 fold and used in the assay. At these sera dilutions maximum sensitivity was observed under the assay conditions. Following incubation with test sera, 2000 fold diluted anti human IgG peroxidase (Sigma) or 500 fold antihuman IgM peroxidase (Sigma) were used for detection of filarial specific IgG and IgM. For subtyping IgG sub groups, 1000 fold

diluted biotinylated antihuman IgG1 (Sigma) or 500 fold diluted biotinylated monoclonal antihuman IgG2 (Sigma) or 1000 fold diluted IgG3 (Sigma) or 2000 fold diluted biotinylated antihuman IgG4 (Sigma) were used followed by addition of 1000 fold diluted extravidin-peroxidase conjugate (Sigma). After incubation, the plates were washed extensively and enzyme activity was measured using o-phenylenediamine (Sigma) (1 mg of the substrate/ml in citrate-phosphate buffer, pH 5·5, with 1 μl of H_2O_2) and absorbance read at 492 nm using an EIA reader (Bio Rad, Richmond, USA). The results were expressed as arbitrary ELISA units by using an internal standard serum kindly provided by Prof. Rick Maizels,University of Edinburgh,UK.

Inhibition of antibodies to PC. A panel of sera were absorbed with 50 μg/ml of PC-BSA [1:1] at 4°C overnight and then for 2 h at 37°C, microfuged at 1200 × g and antibody reactivity to Fil.Cho and PC-BSA were tested by ELISA as described above. For control, another alliquot of the same sera were treated with BSA-g and tested against Fil.Cho and PC-BSA.

Lectin ELISA

Lectin ELISA was performed to test the purity of sugar (Fil.Cho) and protein antigens (Fil. Pro). Con-A (Sigma) was biotinylated by addition of 250 μg of Biotinamidocaproate-N-hydroxysuccinamide ester (Sigma) dissolved in 25 μl DMSO to 1 mg of lectin in 1 ml of bicarbonate buffer (pH-8·5), incubated at RT for 6 h and dialysed extensively in PBS after stopping the reaction by addition of 20 μl of 1 M NH_4Cl. The antigen coated plates were probed with 1000 fold diluted biotinylated Concavalin A for 1 h. followed by washing and addition of avidin peroxidase for 45 min. The enzyme activity was detected as described above for ELISA.

Statistical analysis

Statistical analysis were performed by using Student's 't' test.

RESULTS

Specificity of Fil.Pro and Fil.Cho in ELISA

Since filarial protein antigens were prepared by treatment of an aqueous extract of adult female worms with Sodium meta-periodate ($NaIO_4$), it was essential to ascertain that the oxidation process was complete and that there were no residual carbohydrate epitopes retained in Fil.Pro. Similarly it was essential to ascertain absence of protein epitopes in Fil.Cho that was used as filarial carbohydrate fraction for immunoassays. The ELISA were thus evaluated for specificity of antibodies to filarial proteins and carbohydrate antigens used in the study. For detection of antibodies to parasite carbohydrates earlier investigators had followed either a protocol of *in situ* treatment of antigen coated EIA plates/antigen blotted nitro cellulose membranes by periodate oxidation to inactivate carbohydrate epitopes [13,14] or treatment of glycoproteins in tubes with $NaIO_4$ followed by recovery of protein fraction for use in immunoassays [15,16]. We evaluated these two protocols using two different probes, a lectin for detecting carbohydrate epitopes and IgG4 antibodies for detecting only proteins. The rational was that the binding of Con-A will be significantly decreased on treatment of Fil.Nat with $NaIO_4$ and the binding of filarial specific IgG4 antibodies will remain unaltered on $NaIO_4$ treatment – human IgG4 antibodies are known to bind only to protein epitopes since humans are genetically deficient in eliciting IgG4 antibodies to polysaccharide antigens [17,18]. The results of *in situ* treatment with $NaIO_4$ are shown in Fig. 1a. The binding of biotinylated Con-A to filarial antigen decreased by 70% and the reactivity of IgG4 antifilarial antibodies also decreased by 48% indicating that *in situ* treatment of Fil.Nat coated plates with $NaIO_4$ retained about 30% of carbohydrates residues and more significantly the protocol had affected adversely protein epitopes also since the binding of IgG4 was also found to be decreased (Fig. 1a). Figure 1b shows evaluation of the protocol used in the study. Treatment of Fil. Nat with $NaIO_4$ in tubes resulted in highly significant loss of carbohydrate epitopes as shown by only 7% binding of Con-A to Fil Pro. There was no significant loss of protein epitopes in Fil.Pro since 83% reactivity of filarial IgG4 antibodies was retained after the oxidation. The Fil.Cho coated plates on the other hand reacted very well with Con-A and there was no demonstrable binding of IgG4 antibodies to filarial carbohydrates (Fig. 1b). These results indicate high specificity of both the protein and carbohydrate fractions used in the present study. Every batch of Fil.Pro and Fil.Cho was tested for such specificity before use in immunoassays.

Protein and carbohydrate contents in parasite

The relative contents of carbohydrates and proteins in various developmental stages of *S. digitata* were quantified and the results are shown in Table 1. The adult female and microfilarial extracts were found to be very rich in carbohydrates while the E-S antigens of adult female parasites contained relatively more carbohydrate than protein mass. The adult male worms on the other hand were found to represent a protein dominant developmental stage of filarial parasites. The carbohydrate-protein ratio in males was about 8–10 fold less in comparison to adult female worms or microfilarial stages.

Antibodies to Fil. Pro and Fil.Cho in filariasis

Initially a panel of 20 human Bancroftian filariasis sera were tested for antibodies to Fil. Pro and Fil.Cho. Sera from both mf carriers and those with chronic filarial disease were included for this experiment. The antibody reactivity was probed with second antibodies to human IgM, IgG, IgG1, IgG2, IgG3 and IgG4 and reactivity to Fil.Pro. and Fil.Cho were compared for each of the Ig isotypes – IgG subclasses. No attempt was made to compare the absorbance values between different antibody isotypes for a given antigen. The results are shown in Fig. 2. Filarial IgM, IgG2 and IgG3 antibodies reacted significantly more to Fil.Cho than to Fil.Pro ($P < 0{\cdot}001$ for IgM and IgG2 and $P < 0{\cdot}01$ for IgG3) while reactivity of IgG1 was more skewed towards Fil.Pro and IgG4 antibodies displayed reactivity exclusively to Fil.Pro (Fig. 2).

Association of IgG subclass reactivity with active infection

There was a clear dichotomy in reactivity of IgG2 and IgG3 to Fil.Cho and that of IgG4 to Fil.Pro in the three categories of cases selected across the disease spectrum in human filariasis. Patients with chronic filarial disease and endemic normals were found to have significantly more IgG2 and IgG3 antibodies to Fil.Cho in comparison to microfilariae carriers (Fig. 3a,c). On the other hand Mf carriers produced significantly more IgG4 antibodies to Fil.Pro than endemic normals and patients with chronic filarial disease ($P < 0{\cdot}01$, Fig. 3b). This dichotomy in IgG2 and IgG3 reactivity to Fil.Cho and of IgG4 reactivity to Fil.Pro was not dependent on clinical manifestation since the pattern of reactivity was alike in

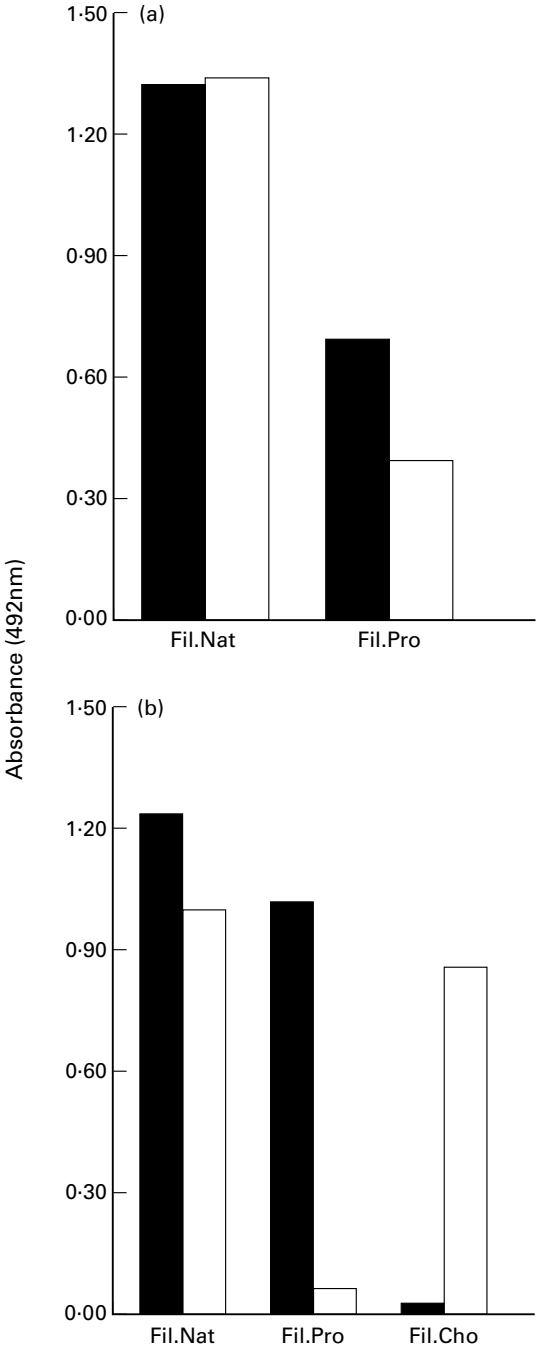

Fig. 1. Testing specificity of Fil.Pro and Fil.Cho by two protocols of filarial protein antigen preparations. a, PBS extract of *S. digitata* (Fil.Nat) coated EIA plates were treated with 25 mM sodium metaperiodate *in situ* and used as filarial protein (Fil.Pro) and was compared with Fil.Nat for reactivity of Con-A (□) or human filarial IgG4 (■). b, In the second protocol Fil. Pro was prepared by treatment of Fil.Nat with 25 mM sodium metaperiodate in tubes and recovered after dialysis and used for coating in EIA. The supernatant after TCA precipitation (of Fil.Nat) was used as carbohydrate fraction (Fil.Cho). Several batches of Fil.Pro and Fil.Cho were tested and a representative experiment on reactivity of Con-A or human filarial antihuman IgG4 are shown.

Table 1. *Setaria digitata*; ratio of carbohydrate and protein contents in adult male, female worms, mf and E-S products

No.	Antigen	mg carbohydrate/mg protein *
1.	Adult females	1·53 ± 0·346
2.	Adult Males	0·216 ± 0·133
3.	Microfilariae	2·42 ± 0·255
4.	Excretory-Secretory product	1·22 ± 0·34

*Mean ± SD of four different batches of antigen preparations.

endemic normals and chronic filariasis cases who are at the two extreme poles of the disease spectrum. The difference rather appeared to be dependent on the status of active filarial infection. When the sera samples were classified according to presence or absence of circulating filarial antigen (CFA, a more definite parameter of active filarial infection than microfilaraemia), individuals with CFA (with or without mf in circulation) were found to possess significantly more IgG4 antibodies to Fil.Pro than to Fil.Cho (Fig. 4b). Those who tested negative for CFA had significantly higher levels of IgG2 and IgG3 antibodies to Fil.Cho (Fig. 4a,c). Although the reactivity of IgG1 was skewed towards Fil.Pro (Fig. 3d) the antibody levels of this subclass was found to be similar to IgG2 and IgG3 -significantly more IgG1 antibodies were found in subjects without active infection than in those with CFA (Fig. 4d). Table 2 shows the ratio of antibody response to Fil.Cho and Fil.Pro in the above investigations – a ratio of >1 indicates a dominant antibody response to Fil.Cho and a ratio of

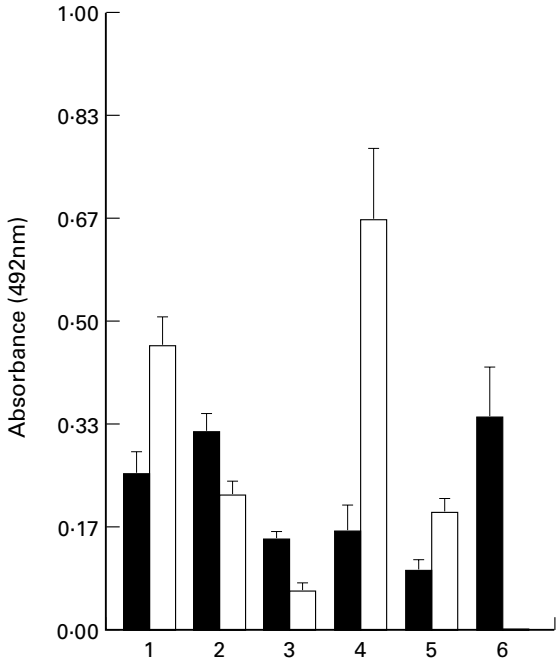

Fig. 2. Human Bancroftian Filarisis: Distribution of Ig isotypes/IgG subclasses of filarial antibodies to Fil. Cho (□) and Fil.Pro (■); probed with Anti-Human IgM (1), Anti-Human IgG (2), Anti-Human IgG1 (3), Anti-Human IgG2 (4), Anti-Human IgG3 (5) and Anti-Human IgG4 (6); Mean ± SEM of 20 filarial sera are shown; 't' test: $P < 0{\cdot}001$ in 1, 3, 4 and 6; and $P < 0{\cdot}01$ in 2 and 5 for antibody reactivity to Fil.Cho and Fil.Pro.

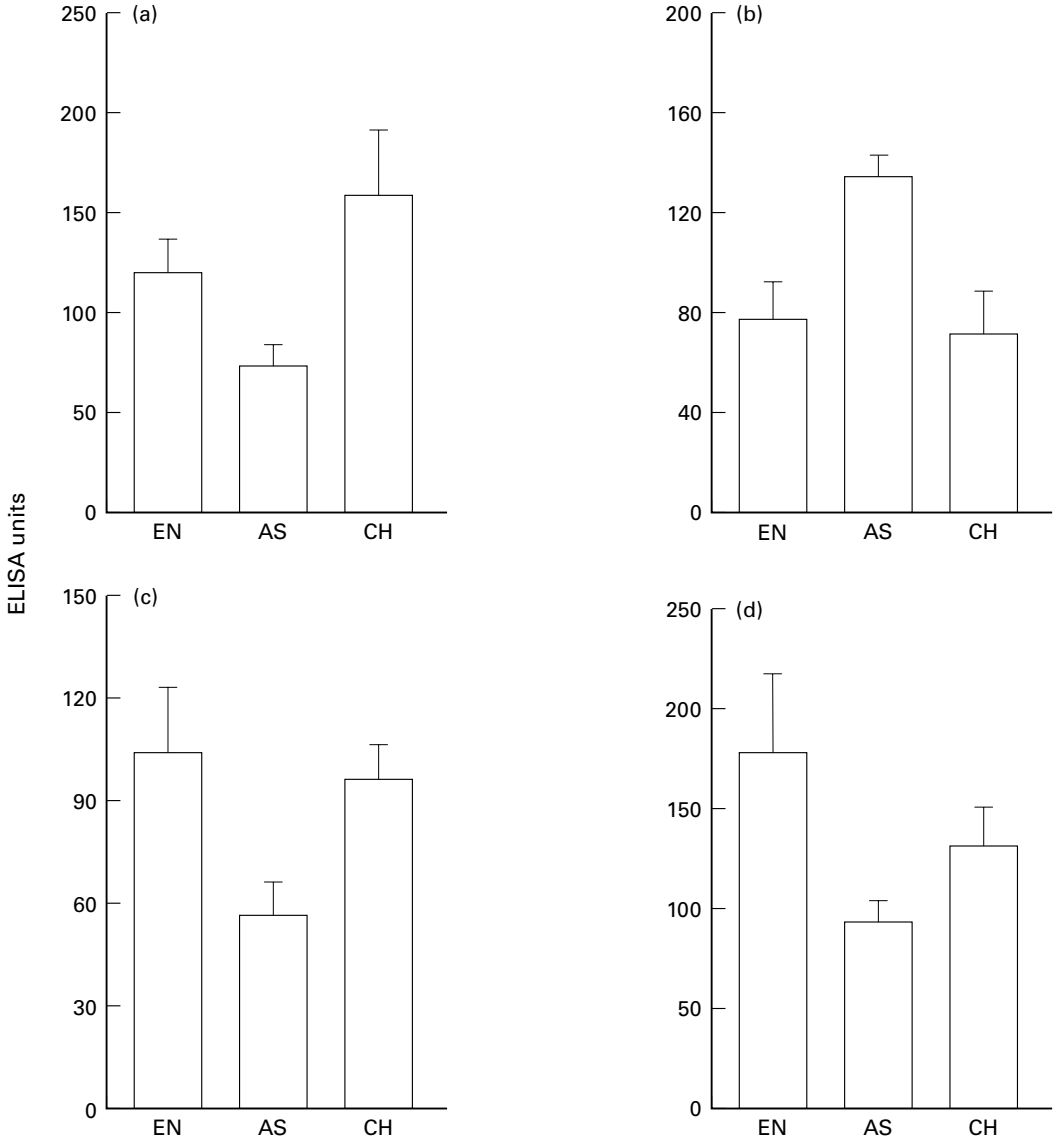

Fig. 3 Antibodies to Fil.Cho and Fil.Pro in three different categories of human Bancroftian filariasis: IgG2 and IgG3 antibodies to Fil.Cho (Fig. 3a,c, respectively) and IgG4 and IgG1 antibodies to Fil-Pro (Fig. 3b,d, respectively) were quantified in Endemic Normals (EN) ($n = 18$ for IgG2 and IgG4; $n = 12$ for IgG3 and IgG1), microfilaraemic, asymptomatic subjects (AS) ($n = 24$ for IgG2 and IgG4; $n = 14$ for IgG3 and IgG1) and patients with chronic filarial disease viz. lymphoedema/elephantiasis (CH) ($n = 11$ for IgG2 and IgG4; $n = 14$ for IgG3 and IgG1); Mean ELISA units ± SEM; 't' test: (a) EN *versus* AS, $P < 0{\cdot}01$ and CH *versus* AS, $P < 0{\cdot}05$; (b) EN *versus* AS, $P < 0{\cdot}01$, CH *versus* AS, $P < 0{\cdot}01$; (c) AS *versus* CH, $P < 0{\cdot}01$, AS *versus* EN, $P < 0{\cdot}05$; (d) EN *versus* AS, $P < 0{\cdot}05$.

< 1 indicates a dominant response towards Fil.Pro. The ratio also precisely indicates the degree of skewing of antibody response to one or the other determinant. Thus a ratio of 4·15 for IgG2 antibodies in human filarial sera indicates the extreme dominance of this isotype to Fil.Cho and a ratio of 0·005 for IgG4 indicates its extreme dominance in recognizing protein epitopes.

Antibodies to Fil Cho recognize non-PC determinants

Phosphorylcholine (PC) residues are known to be terminally linked to filarial carbohydrates [19] and the Fil.Cho preparation used in the present study was found to contain PC determinants when tested with a standard MAb to PC (data not shown). Since antibodies to PC are known to be present in human filariasis [20], it was essential to investigate what proportion of antibodies to Fil.Cho quantified in the present study was directed towards PC. A panel of sera were absorbed with PC-BSA and tested for antibody reactivity to Fil.Cho as well as to PC. The results are shown in Fig. 5. The sera absorbed with PC-BSA lost only about 10% reactivity when tested against Fil.Cho while the same sera, as expected, lost more than 95% reactivity when tested against PC-BSA indicating thereby that nearly 90% of the antibodies reacting to Fil.Cho were recognizing non-PC determinants present in Fil.Cho.

DISCUSSION

Carbohydrates have been generally perceived to be decoy antigens diverting the immune response away from protective protein

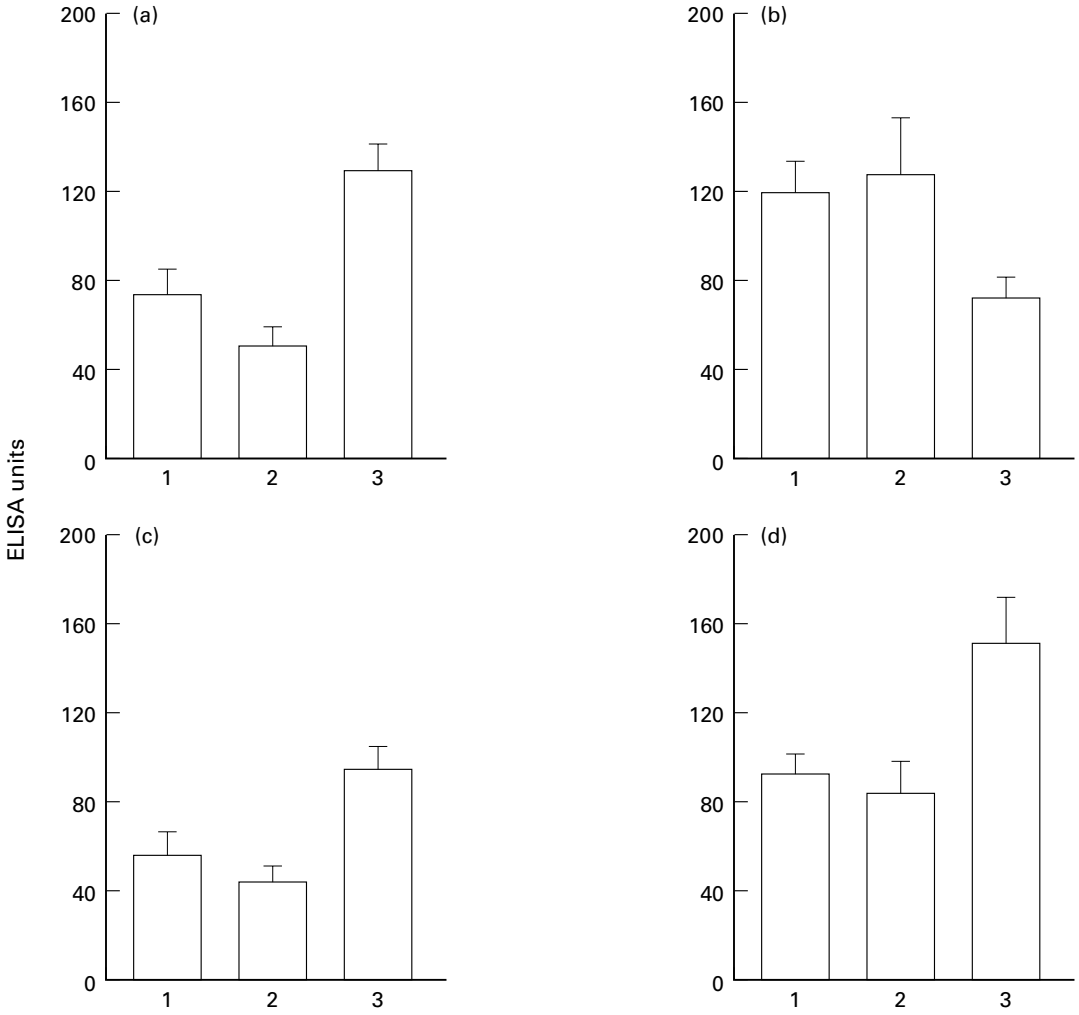

Fig. 4 Antibodies to Fil.Cho and Fil.Pro and association with circulating filarial antigen (CFA) and microfilaraemia in Bancroftian filariasis: Human filarial sera positive for mf and CFA (1) or positive for CFA and negative for mf (2) and or negative for both mf and CFA (3) were tested against Fil.Cho coated plates and probed with Anti-human IgG2 and IgG3 (a and c, respectively); the same set of sera were tested against Fil.Pro and probed with Anti-human IgG4 and IgG1 (b and d, respectively); Mean ELISA units ± SEM; 't' test: (a) 1 *versus* 3, $P < 0{\cdot}01$; 2 *versus* 3, $P < 0{\cdot}001$. (b) 1 *versus* 3, $P < 0{\cdot}01$; 2 *versus* 3, $P < 0{\cdot}05$. (c) 1 *versus* 3, $P < 0{\cdot}05$; 2 *versus* 3, $P < 0{\cdot}01$. (d) 1 *versus* 3, $P < 0{\cdot}05$; 2 *versus* 3, $P < 0{\cdot}05$. (1 mf + ve CFA + ve: $n = 21$ for IgG2 and IgG4 and $n = 14$ for IgG1 and IgG3; 2 mf-ve CFA + ve: $n = 13$ for IgG2 and IgG4 and $n = 14$ for IgG1 and IgG3; 3 mf-ve CFA –ve: $n = 38$ for IgG2 and IgG4 and $n = 25$ for IgG1 and IgG3).

epitopes in helminthic diseases [1]. Antibodies of IgM and IgG2 isotypes (directed towards carbohydrate determinants) have been suspected to block responses to schistosomula by granulocytes, which are guided by antibodies of other isotypes in ADCC [7]. More recently it has been demonstrated in murine schistosomiasis that parasite carbohydrates polyclonally stimulate CD5 + B-lymphocytes to produce IL-10 which down regulate protective Th1 type of immune responses [21,22]. These investigations tend to indicate a role for carbohydrates in mediating immune deviation and assisting parasites for prolonged survival in infected hosts.

Several findings reported in literature point towards a host protective role for antibodies to carbohydrates in filariasis. Anti-microfilarial immunity is characterized by presence of antibodies to microfilarial sheath (mostly IgM and IgG2) both in Brugian and Bancroftian Filariasis and antisheath antibodies recognize mostly carbohydrate determinants on the microfilarial sheath [23–25].

Table 2. EIA: Antibodies to Fil. Pro and Fil. Cho of *S. digitata* in human Bancroftian filariasis.

Isotype/Sub-class of filarial antibody	Ratio*	% of samples with ratio of >1
IgG	0·682	10·0
IgM	1·822	100
IgG1	0·445	10·0
IgG2	4·155	100
IgG3	1·92	90·0
IgG4	0·005	0

* Ab to Fil.Cho/Ab to Fil.Pro; mean ratio of 20 sera (10 mf carriers and 10 chronic filarial patients)

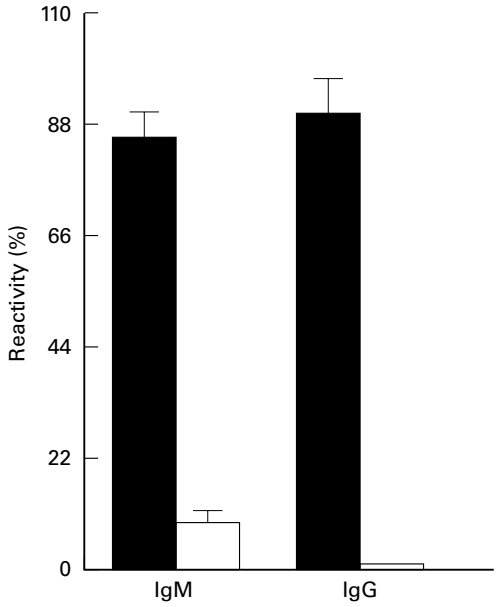

Fig. 5 Antibodies to Fil.Cho recognize non-PC determinants. Sera were absorbed with PC-BSA and tested for reactivity to Fil.Cho (■) and PC-BSA (□). % Mean ± SEM reactivity ($n = 4$) was calculated with reference to values observed in native unabsorbed sera.

Similarly, antilarval immunity in human filariasis is characterized by presence of antibodies directed towards surface of infective larvae. Such antibodies, mostly IgM and IgG2 but not IgG4 presumably recognize carbohydrate epitopes since attempts to demonstrate reactivity of these antibodies to radiolabelled surface protein antigens have been unsuccessful [26,27]. Furthermore, presence of antibodies reacting with the surface of infective larvae is a consistent feature in experimental animals immunized with γ-irradiated infective larvae (a procedure that induces protective immunity) and attempts to implicate immune response to protective protein epitopes in these experiments have not been successful [28]. In this context, it is not surprising that the findings of the present study point towards a protective role for antibodies to carbohydrates in human bancroftian filariasis. Significantly low levels of IgG2 and IgG3 antibodies to Fil.Cho were observed in individuals harbouring circulating filarial antigens (CFA, a definitive parameter of active infection) in comparison to those devoid of CFA. IgG4 antibodies to Fil.Pro on the other hand were found to be high in CFA positive cases. Significantly high levels of IgG2 and IgG3 antibodies to filarial carbohydrates were observed in chronic filariasis cases and 'putatively immune' Endemic normals in comparison to mf carriers indicating that although higher levels of antibodies to Fil.Cho could be associated with absence of active infection, it could have no bearing on clinical manifestations of filariasis since endemic normals and chronic filariasis are at the two extreme poles of the disease spectrum.

A nonfractionated crude preparation of filarial carbohydrates which is free of protein epitopes has been used in the present study. Inhibition with PC-BSA has indicated that about 85–90% of both IgG and IgM antibodies reacting to Fil.Cho essentially recognize non-PC determinants. Further fractionation of Fil.Cho and using structurally defined fractions of filarial carbohydrates could lead to better understanding of the immuno genicity of carbohydrates and their role in protective immunity in human filariasis.

Apart from mediating ADCC to infective larvae or microfilariae, antibodies to filarial carbohydrate determinants can be expected to operate through other mechanisms in inducing protective immunity in helminthic infections. The immunological hyporesponsiveness observed in both filariasis and schistosomiasis has been demonstrated to be mediated by IL-10 [22,29]. In schistosomiasis, parasite carbohydrate determinants (Lacto-N-fucopentose-III) have been shown to be responsible for polyclonal stimulation of CD5 + B-lymphocytes for production of large quantities of IL-10 [20]. Antibodies to parasite carbohydrates could neutralize such down regulating signals and confer protective immunity. While down regulation of T-cell proliferation and IFN-γ production have been demonstrated in filariasis [29], carbohydrate determinants analogous to lacto-N-fucopentose-III of Schistosoma are yet to be identified in filarial parasites. However, it has been demonstrated that adult female worm antigens suppress IFN-γ production by T-cells and adult male worm antigens stimulate higher levels of IFN-γ in mf carriers [30]; these findings reported a few years ago can be interpreted in the context of relative protein–carbohydrate contents in male and female worms observed in the present study. It is possible that carbohydrate rich adult female parasites more readily suppress IFN-γ (through induction of IL-10) than protein rich adult male parasites. We are currently testing this possibility by using Fil.Cho and Fil.Pro for induction of IL-10 and IFN-γ by lymphocytes in human filariasis.

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